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DEPARTMENT OF THE ARMY
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OUR EXPERIENCES WITH DISINFECTION BY MEANS
OF ETHYLENE OXIDE

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181-186.

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In medical work, the need has been constantly growing for complete sterilization of various apparatus and instruments made from artificial substances or other material not capable of withstanding temperatures above 80°C. It has become obvious that many necessary pieces of equipment used in modern medical practice cannot be sterilized by traditional means, namely in autoclaves. Disinfection with formaldehyde is not suitable because this gas has more a bacteriostatic than a bactericidal effect, and is not effective against sporulating microorganisms. Therefore, present-day attention is directed towards the so-called cold gas sterilization, in which gaseous disinfecting substances are used at lower temperatures (ethylene oxide, betapropiolactone, peracetic acid, and the like). The aim is not to replace the classical and well established hot sterilization, but mainly to find a method of sterilizing, and possibly disinfecting materials which cannot withstand high temperatures.

Ethylene oxide (further called EO) $\text{CH}_2\text{-CH}_2$ is a gaseous substance with a boiling point of 10.8°C and a solidification point of -111.3°C, with a specific gravity of 0.8838. It is very well soluble in water and in organic solvents. Under pressure it can spontaneously polymerize, whereby the polymer is explosive. Mixed with oxygen (with air) it explodes, although the mixture with carbonic acid in a proportion of 1:9 is absolutely non-explosive and non-inflammable. Its manufacture is relatively simple and inexpensive. It is nowadays constantly used in the chemical industry as alkylating agent. Some authors also explain the mechanism of the bactericidal effect of EO by alkylation. According to the alkylation theory, EO replaces the labile H atom with the hydroxyethyl

group ($-\text{CH}_2-\text{CH}_2-\text{OH}$) and thereby inactivates many reactive groups, e.g. $-\text{NH}_2$, $-\text{SH}$, $-\text{OH}$.

EO is a moderately toxic and irritating substance. It is often used for conservation of food products, whereby, however, their biological value is lowered because EO disturbs the whole vitamin B complex and other biologically effective substances. If the objects, after disinfection, are removed from the EO, the remainder of this gas evaporates quickly from them. Only rubber and some plastic substances, in which EO is soluble, release EO slowly, over several hours. According to data in the literature, EO has good penetrating ability, and it is said that thereby also its bactericidal effect potentiates.

The explosive range of EO is found in a mixture with air above 3%. By addition of an inert gas in a proportion of maximally 12% of weight, the mixture becomes non-inflammable, however by the increase of the proportion of the inert gas the bactericidal effect of EO is substantially lowered. The mutual dependence of the time of exposure and the concentration of the gas is in an invert proportion. With constructionally complicated objects, the time of exposure is prolonged up to 24 hours. This disadvantage (of the action requiring a long time) can partly be eliminated by use of higher pressure in sterilization. An important factor with the utilization of EO for disinfection is the humidity of the environment; the optimum is between 20 and 40% of relative humidity. One can therefore not sterilize lyophilizates with EO, and on the other hand the sterilization effect is also uncertain in a very humid environment.

In order to verify data from the foreign literature, we performed a more extended control of the sterilizing effect of EO in a prototype apparatus, similar to an autoclave, which had been manufactured in the National Enterprise "Chirana".

Table 1. Effect of Ethylene Oxide on *Staph. Aureus*, *B. Subtilis*, and *Mycobact. Phlei*, at a Temperature of 19°C.

1) Exposure in hours	0.5 h				1 h				2 h				4 h			
	3	4	5	6	3	4	5	6	3	4	5	6	3	4	5	6
3) <i>Staph. aureus</i>	17	17	17	17	17	17	17	17	17	17	17	17	17	17	17	17
4) <i>B. subtilis</i>	17	17	17	17	17	17	17	17	17	17	17	17	17	17	17	17
5) <i>Mycobact. Phlei</i>	17	17	17	17	17	17	17	17	17	17	17	17	17	17	17	17

KEY: 1. Exposure in hours; 2. Relative Humidity %; 3. Total of samples; 4. Positive culture; 5. Control.

Table 2. Effect of Ethylene Oxide on Staph. Aureus, B. Subtilis, and Mycobact. Phlei, at a Temperature of 65°C.

1) Exposure in hrs.	0 atm				1 atm				2.5 atm				6.5 atm			
	Staph. aureus	B. subtilis	Mycobact. phlei	Rel. hum.	Staph. aureus	B. subtilis	Mycobact. phlei	Rel. hum.	Staph. aureus	B. subtilis	Mycobact. phlei	Rel. hum.	Staph. aureus	B. subtilis	Mycobact. phlei	Rel. hum.
3	17	17	17	60	17	17	17	60	17	17	17	60	17	17	17	60
5	17	17	17	60	17	17	17	60	17	17	17	60	17	17	17	60
7	17	17	17	60	17	17	17	60	17	17	17	60	17	17	17	60
5) Control	17	17	17	60	17	17	17	60	17	17	17	60	17	17	17	60

KEY: 1. Exposure in hours; 2. Relative humidity; 3. Total of samples; 4. Positive culture; 5. Control.

Methodics

We used EO in a mixture with CO₂ in a proportion of 9.6% EO and 90.4% CO₂. The analysis was performed by means of gas chromatography. With the quoted concentration, we determined the following amounts of grams of EO per 1 m³ with given temperature and pressure:

20°C		65°C	
Pressure in atm	EO in g/m ³	Pressure in atm	EO in g/m ³
0	176	0	152
1	353	1	304
2.5	617	2.5	533
6.5	1328	6.5	1142

Sterilization was performed at a temperature of the gas of 19°C and 65°C and at a pressure of 0 atm, 1 atm, 2.5 atm, and 6.5 atm and with 3, 5, and 7 hours exposure. The relative humidity ranged between 30 and 98%. For contamination of the carrier we used the following microbes: Staphylococcus aureus with 25/48 UEM, 18 hours cultures in an amount of approximately 24.10 with 1 ml of aqueous suspension; furthermore B. subtilis 8/58 UEM in a well sporulated 6 day culture in an amount of approximately 14.10⁷ in aqueous suspension; and lastly Mycobacterium phlei 11/49 UEM. The drying of the germs on the carrier was done in a thermostat at 37°C over 3 hours. After contamination we separated the carriers into sterile paper envelopes, which were then put into Schimmelbusch drums. These drums were put into a special sterilizing apparatus. As carriers were used ordinary surgical silk thread, surgical sillon thread, strong surgical silk thread, a small

PVC tube, a small tygon tube, a PVC catheter, a knot from novodur, a tygon catheter, neoplex, a rubber drain, a rubber catheter, a black urethral catheter, surgical rubber gloves, an injection needle with mandrel, plexi-glass (polyacrylate), an ordinary tampon, and an injection syringe.

The control of sterilization itself was performed in a simple manner. After opening the sterilizing cylinder, we transferred the carriers into suited culture media (MP bouillon, Sul media), where they were left for 30 minutes. Then we took the carriers sterily out of the culture medium and cultured the media in the thermostat at 37°C. Then we prepared the used carriers again for further experiments. The results were read with *Staph. aureus* after 48 hours, with *B. subtilis* and *Mycobact. phlei* after 7 days. At the same time, we performed a control of the contamination of the carriers and a control of the sterility of the culture media.

Results

The reproduced tables show that a sterilizing effect of EO could not be shown, not even at a temperature of 65°C and the relatively high pressure of 6.5 atm. It was further shown that even a considerably prolonged exposure time (i.e. 7 hours) does not suffice for complete sterilization. Striking is the difference between carriers contaminated with vegetative forms of microbes and carriers contaminated with sporulating bacilli. This difference is in agreement with the known properties of the mentioned bacteria, particularly as far as their resistance to varying high temperatures is concerned. We have cultured *B. subtilis* from 15 out of 17 carriers after exposure to EO at a temperature of 65°C, a pressure of 3.5 atm, and after 5 hours exposure, which roughly corresponds to the positive cultures with the controls. By contrast, *Staph. pyogenes* proved to be much more sensitive to the action of EO under the same conditions, for it could not be established even from one of 17 contaminated carriers. The same effect on staphylococci was achieved with a pressure of 2.5 atm already, although with a temperature of 65°C and 5 to 7 hours exposure. Striking is the small bactericidal effect of EO at a temperature of 19°C, even with a pressure of 6.5 atm and 5 to 7 hours exposure. Under these conditions, the growth of *B. subtilis* is absolutely uninfluenced, even when staphylococci and mycobacteria could be shown in the culture (although in relatively few cases).

Discussion

On the basis of the quoted results we can therefore say that EO does not show a sterilizing effect against the tested microbes at temperatures up to 65°C. That the used pressure does not make any difference. By prolonged exposure and a temperature of 65°C the vegetative forms of vegetative microorganisms, in our case pyogenic staphylococci, can be destroyed. It is, however, an open question what

actually causes this bactericidal effect, whether ethylene oxide or the prolonged application of this high temperature. The results of sterilization at a temperature of 19°C are certainly influenced by the fact that it had not always been possible to achieve the desirable relative humidity (in these experiments, the relative humidity ranged between 5% and 98%). In the experiments with a temperature of 65°C, the fluctuation of the relative humidity was somewhat more favorable, for the maximum reached was 62%.

For a deeper analysis of the disinfecting effect of EO it would in our opinion be necessary to test more kinds of microbes than have been used in our experiments so far. However, for technical reasons we have not been able to increase the already high number of experiments and cultivations (1360). If we evaluate the results according to the used carriers, we see that the material from which they were made was not as important as their type. E.g., in the relatively small percentage of positive cultures of *Staph. pyogenes* and *Mycobact. phlei* with relatively high temperatures and prolonged exposure there had been used relatively frequently the urethral catheter, the injection needle, and the thin PVC catheter. It is therefore obvious that even the penetrating ability of EO, which many authors have so very much stressed, does not ensure a dependable sterilization.

Summary

The sterilizing effect of ethylenoxyd on three kinds of microbes, *Staphylococcus pyogenes*, *Mycobacterium phlei*, *Bac. subtilis*, has been examined, using the temperatures of 19°C and 65°C and with the pressure of 0 atp., 1 atp., 2.5 atp. and 6.5 atp. The time of exposure was 3 hours, 5 hours and 7 hours. As microbe carriers 17 different instruments, mostly made of plastics, were used. From the experiments it is obvious that ethylenoxyd has no sterilization ability. Bacteria killing effects of ethylenoxyd on the vegetative forms of bacteria are obviously connected with the temperature used (i.e. 65°C) and with the long time of exposure (5 to 7 hours). Therefore we consider ethylenoxyd to be a disinfectant under favorable conditions if the temperature of 65°C is used for the duration of several hours. Using this gas by lower temperatures does not guarantee a reliable disinfection and is therefore not suitable for field conditions.

BIBLIOGRAPHY

- Wall, E. L., Dornbusch, A. C.: *App. Microb.* 9, 4, 1961.
Freeman, M. A. R., Barwell, C. F.: *J. Hyg.* 58, 3, 1960.
Himmelfarb, P., Read, R. B.: *App. Microb.* 9, 6, 1961.

Kelsey, J. C.: J. Clin. Path. 14, 59, 1961.

Koernlein, M., Seibel, M.: Die neue Verpackung (New Packaging), 10, 1959.

Lowbury, E. J. L.: Brit. J. Industr. Med. 8, 1951.

Privora, M.: Zpravy z epidemiologie a mikrobiologie (Reports on Epidemiology and Microbiology), 1, 1962.

Schley, G. D. and collab.: App. Microb., 8, 1960.

Sykes, G.: J. App. Bact. 25, 1, 1962.

Sykes, G.: Disinfection and Sterilization, London E., F. N. Spon Ltd., 1958.

Walter, C. W., and collab.: JAMA, 13, 1959.